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Seasonal cycle of copper speciation in Gullmar Fjord, Sweden

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Abstract

The chemical speciation of dissolved Cu was investigated by voltammetric methods in Gullmar Fjord, Sweden, over the course of a year from September 1996 until August 1997. Sampling was carried out on a roughly monthly basis, with an intensive survey carried out in May 1997. Surface water temperatures ranged from −1 to 22°C, whereas bottom waters in the fjord were approximately 6°C throughout. Macronutrient concentrations in the fjord during the period of the survey were investigated independently by the Göteborgs och Bohus läns Vattenvårdsförbund (Water Quality Association of Göteborg and Bohus). Surface phosphate concentrations were highest in early spring with low levels (<0.1 μmol kg^{-1}) over the late spring and summer. Nitrate and silicate showed a similar pattern to phosphate with the exception of high concentrations encountered in surface waters when low salinity plumes caused by runoff were encountered. A period of calm, sunny weather in January 1997 saw the initiation of the spring bloom some 2 months earlier than usual. Dissolved Cu speciation was dominated by organic complexation (over 99.8%) throughout this study. Strong Cu binding ligands (log K $>$ 12.5) were not detected during the winter or early spring and could be related to the temperature-related seasonal appearance of the cyanobacterium *Synechoccocus* in these waters. The appearance of the strong Cu ligands led to a decrease in the concentration of free copper, resulting in a seasonal cycle for free copper in the fjord. This is the first study to examine Cu speciation over an annual cycle in a coastal environment.

The distribution and chemical speciation of trace metals in the upper water column plays an important role in the community structure and physiology of phytoplankton (Sunda 1994). Speciation is important because only particular chemical forms of a given metal may be biologically available. In the upper water column, the speciation of many biologically active trace metals is controlled by complexation with strong organic ligands (Bruland et al. 1991). In general, when a metal is complexed by an organic ligand, the metal becomes less biologically available because the free metal ions are the most labile to the biota (Sunda 1994; Campbell 1995). Organic complexation also can play an important role in the biogeochemical cycling of these elements in the upper ocean. For most elements, organic ligand concentrations are highest in the euphotic zone (Bruland et al. 1991), which suggests a recent biological source.

Complexation is of particular importance for Cu because it is both an essential micronutrient and extremely toxic. Field studies performed in open ocean and in coastal waters have determined that Cu is complexed by low concentrations of strong ligands with further trace metal buffering by higher concentrations of weaker ligands (van den Berg et al. 1987; Coale and Bruland 1990; Sunda and Huntsman 1991; Moffett 1995). These strong ligands frequently are found at concentrations slightly in excess of the ambient copper concentration in seawater and have conditional stability constants of $10^{12.5}$ mol L^{-1} or greater. Currently there is no structural or functional chemistry information on these ligands, and they are generally referred to as ligand class 1. The weaker ligands that are also detected in seawater are similarly classified as ligand class 2 and have conditional stability constants ranging from 10^{10} to 10^{12} mol L^{-1}.

Fieldwork in the Sargasso Sea has shown a strong link between the presence of strong Cu complexing ligands (class 1) and the cyanobacterium *Synechoccocus* (Moffett et al. 1990; Moffett 1995). Laboratory cultures of *Synechoccocus* isolated from the Sargasso Sea have also been found to produce strong Cu binding ligands when under Cu stress (Moffett and Brand 1996). The growth of *Synechoccocus* is very sensitive to Cu, with growth rates reduced at free copper concentrations as low as 10 pmol L^{-1} ($[Cu]_f = 10^{-11}$ mol L^{-1}) (Brand et al. 1986). *Synechoccocus*, in ambient open-ocean seawater (1–5 nmol L^{-1} Cu) if there were no organic complexation of Cu, would be significantly inhibited. Thus *Synechoccocus* benefits from the production of class 1 ligands in the presence of elevated Cu concentrations. It has been suggested that in the natural environment, *Synechoccocus* produces class 1 ligands to create conditions more favorable for its growth. Fieldwork in Massachusetts harbors subject to anthropogenic Cu saw a strong inverse relationship between $[Cu]_f$ and cell densities of *Synechoccocus*, as expected from laboratory studies (Moffett et al. 1997).

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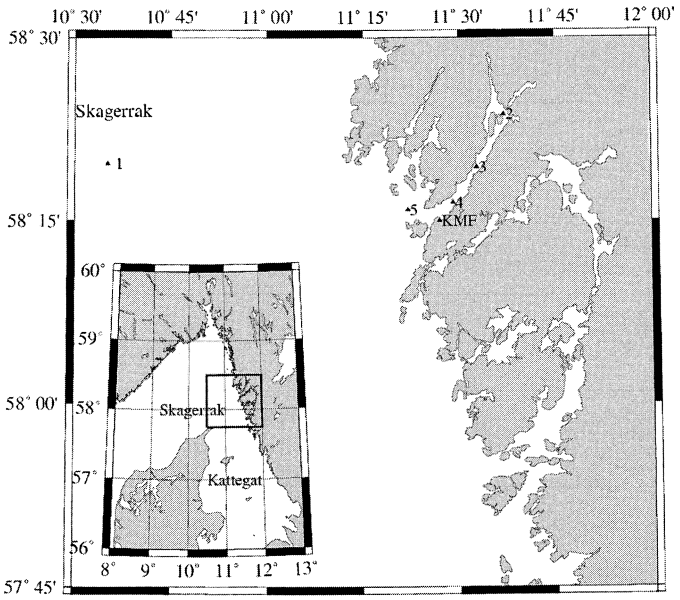

Fig. 1. Map of study region, Gullmar Fjord, and regional oceanographic setting (inset). Also shown are the positions of the sampling stations occupied during the course of this work.

Gullmar Fjord is a relatively pristine fjord situated on the west coast of Sweden (Fig. 1). The fjord is 30 km long and 3 km wide. The main part of the fjord is straight with steep rocky shores. It has a maximum depth of 120 m and a sill depth of 45 m. The water body in the fjord is always stratified and normally consists of three layers, each of different origin (Lindahl and Hernroth 1983): high-salinity water from the North Sea in the deep basin of the fjord (below 50 m depth, salinity 34–35), water from the Skagerrak forming an intermediate layer (20–50 m, salinity 31–33), and water originating from the Baltic Sea comprising the top layer (salinity 18–30). Wind and barometric pressure changes determine the occurrence and vertical distribution of the different water masses and a thermocline is more or less pronounced from May until September, normally at 15 to 20 m (Lindahl and Hernroth 1983). Exchange of the water in the fjord above the sill depth with water from outside is driven by vertical movements of the halocline outside the fjord and occurs within less than a month (Rydberg 1977; Liljebladh and Thomasson 2001). The annual mean freshwater runoff from land into the fjord is 22 m^3 s^{-1}, with the main river, Örekilsälven, located at the head of the fjord. This runoff can contain a high humic load, which can turn the upper water layer brown. The deep water in the fjord has an Atlantic origin and is normally renewed once a year in one or several pulses during late winter or early spring. Occasionally, this renewal does not take place and the bottom waters can become suboxic or anoxic (Lindahl and Hernroth 1983; Liljebladh and Thomasson 2001).

The spring bloom in Gullmar Fjord typically starts in March, lasts 2–3 weeks, and is dominated by diatoms (Lindahl and Hernroth 1983). Prior to the start of the bloom, macronutrient concentrations are at their highest as winter mixing brings nutrients to the surface and land runoff is high. The spring bloom begins despite occasional ice cover in the fjord (Lindahl and Hernroth 1983). In summer, surface waters are depleted of macronutrients, resulting in almost oligotrophic conditions. The autumn bloom is typically dominated by a large dinoflagellate bloom (Lindahl and Hernroth 1983). *Synechoccocus*-type cyanobacteria are found in the Skagerrak and Gullmar Fjord year round, and show a seasonal abundance with maximum concentrations in summer (Karlson and Nilsson 1991; Kuylenstierna and Karlson 1994).

Table 1. Location of sampling stations in and around Gullmar Fjord.

Station	Maximum depth (m)	Latitude	Longitude
1 Skagerrak	350	58°19.7′N	10°29.1′E
2 Björkholmen	61	58°23.7′N	11°37.1′E
3 Alsbäck/Djuphålan	118	58°19.4′N	11°32.8′E
4 Ingela's	63	58°16.5′N	11°29.1′E
5 Sill/Inloppet	39–52	58°15.9′N	11°22.0′E
6 KMF	50	58°27.0′N	11°27.0′E

KMF, Kristineberg Marine Research Station.

The aim of the present work was to measure Cu speciation over an annual cycle in the Swedish west coast and, in particular, to examine the effect of seasonal *Synechoccocus* abundance on Cu speciation.

Methods

Sampling stations—The location of the sampling stations used in this study can be found in Table 1 and Fig. 1. Four stations within the fjord were occupied at various times throughout this study (Table 2). The station at Björkholmen is also the site of monthly sampling by the Göteborgs och Bohus läns Vattenvårdsförbund. Alsbäck/Djuphålan is located at the deepest part of the fjord. A station was also occupied at the entrance to the fjord, Sill/Inloppet, in the shallowest part of the fjord. The main sampling station was located at Ingela's, located close to the Kristineberg Marine Research Station (KMF). This station was occupied on a monthly basis and formed the key site for the study of the annual cycle. All sampling in Gullmar Fjord was undertaken from the RV *Arne Tiselius*, which is based at KMF. One sample was collected in October 1996 in the Skagerrak adjacent to Gullmar Fjord from the University of Göteborg vessel the RV *Skagerak.*

Water sampling—Prior to seawater sampling, a CTD device equipped with a fluorometer was lowered down through the water column to obtain vertical profiles of salinity, temperature, and chlorophyll fluorescence. Seawater samples were taken with precleaned GoFlo samplers (8 liters) mounted on a 6-mm Kevlar hydrowire. At each station, samples were collected from the Kevlar wire at various depths in the upper 50 m of the water column.

Immediately upon recovery, the GoFlo sampler was wrapped in plastic bags and mounted on a bottle rack. All handling of the GoFlo samplers was performed while wearing plastic gloves. Seawater samples were then drawn into

Table 2. Cu speciation data from Gullmar Fjord Sep 96–Aug 98.

Date	Location	Depth (m)	T (°C)	S	DCu (nmol L^{-1})	Log K_1	L_1	Log K_2	L_2	pCu	*Synechoccocus** (M cells L^{-1})
13 Sep 96	KMF	4	14.8	32.1	5.0	12.9±0.4	5.0±1.0	10.5±0.3	82±8	12.36±0.35	31±17
		35	13.6	34.0	5.0	—	—	12.13±0.22	32.7±2.4	12.87±0.23	
2 Oct 96	Skagerrak	10	—	—	3.0	13.0±0.5	4.8±0.3	10.5±0.3	68±10	12.88±0.46	7.3±1.7
24 Jan 97	KMF	35†	6.7	33.9	4.7	—	—	10.8±0.1	43±8	11.72±0.21	0.4±0.2
17 Mar 97	Ingela's	5	3.5	26.74	13.1	—	—	11.3±0.3	115±14	12.19±0.32	0.08±0.12
	Sill/Inloppet	5	3.7	27.62	12.3	—	—	11.3±0.4	122±19	12.25±0.43	
24 Mar 97	Ingela's	5	3.4	26.20	10.9	—	—	11.43±0.15	92±3	12.30±0.15	0.2±0.2
	Sill/Inloppet	5	3.4	24.84	18.5	—	—	11.1±0.4	115±19	11.82±0.43	
13 May 97	Ingela's	10	8.2	32.15	4.6	13.06±0.16	18±2			13.52±0.20	4.1±1.4
17 May 97	Ingela's	10	10.5	31.96	7.06	—	—	11.9±0.8	55±9	12.73±0.82‡	8.3±2.3
		30	6.8	33.52	4.21	13.10±0.08	27±12	10.6±0.3	29±3	13.84±0.45	2.1±1.2
19 May 97	Ingela's	8	10.8	25.14	5.55	13.24±0.81	14±3	10.9±0.4	45±5	13.44±0.82	9.5±2.2
29 May 97	Björkholmen	5	11.8	21.09	3.88	—	—	11.84±0.18	86±5	13.17±0.19§	
		30	5.8	33.35	4.77	—	—	11.50±0.24	67±7	12.62±0.26§	
	Alsbäck	5	11.9	20.95	6.4	—	—	12.4±0.2	32±2	13.00±0.21§	
		30	6.7	33.47	2.32	—	—	12.02±0.11	53±2	13.36±0.12§	
	Ingela's	5	11.2	21.77	8.63			12.08±0.35	36±3	12.58±0.36§	0.8±0.3
		30	6.9	33.52	9.43	—	—	12.15±0.21	52±3	12.80±0.22§	
13 Jun 97	Alsbäck	10	11.7	24.23	11.70						
	Ingela's	10	13.2	23.28	8.77	14.18±0.44	9.1±1.1	11.9±0.4	38±6	13.43±0.40	72±19
	Sill/Inloppet	10	12.8	22.89	7.10						
4 Jul 97	Sill/Inloppet	7	15.3	26.63	2.02						
		20	10.5	33.77	2.20						
	Ingela's	7	14.6	27.43	4.14	13.8±0.2	7±1	11.3±0.2	14±3	13.66±0.24	53±17
		20	10.7	32.91	7.35	13.75±0.17	7±2	11.5±0.2	31±3	12.89±0.28	23±7
	Alsbäck	5	16.1	24.79	5.77						
		50	6.5	33.75	2.40						
	Björkholmen	4.5	17.1	22.51	5.4						
29 Aug 97	Björkholmen	18.4	15.6	31.01	5.9						
	Alsbäck	6	21.4	22.17	8.6						
		20	15.3	31.39	5.2						
		50	7.7	33.70	6.8						
		90	6.0	34.32	3.2						
	Ingela's	8	21.2	22.92	6.7	13.7±0.2	10±1	11.5±0.2	28±3	13.45±0.21	97±23
		28	13.1	32.93	7.7						
17 Aug 98	Alsbäck	5	16.9	24.05	11.7	—	—	11.74±0.18	92±12	12.58±0.22	
		40	13.0	32.49	10.6	13.6±0.2	12±2	10.7±0.2	72±3	12.87±0.25	
		70	6.3	33.99	6.6	—	—	12.2±0.2	68±6	13.17±0.22	

* Cell counts for *Synechococcus* are expressed as millions of cells per liter.
† Ice cover on fjord, no surface sample.
‡ Large thiol interference in SA titration.
§ Using two ligand-fitting approaches, no convergence for solution.

500-ml acid-cleaned polyethylene bottles, double bagged (Minigrip, Ziplok), and placed in the dark at 4°C. Seawater was filtered over acid-cleaned Nuclepore membrane filters (47 mm, 0.4 μm) mounted in all-Teflon filter holders (Savillex) in a class-100 laminar airflow bench.

Samples for total dissolved trace metals were acidified with 1 ml quartz-distilled HCl (hereafter abbreviated to Q-HCl) per liter of sample and stored for at least 1 week prior to analysis. Samples for competitive ligand exchange–cathodic stripping voltammetry (CLE-CSV) were run at natural pH, within 24 h of collection.

All plasticware used in this work was extensively acid cleaned before use. All solutions were prepared using 18 MΩ Milli-Q water (Millipore system). High-purity Q-HCl (6 mol L^{-1}) was made by redistillation of Merck trace metal–grade acids in a quartz subboiling still (Kuehnen et al. 1972). Ultraclean ammonium hydroxide was purchased from J. T. Baker.

Macronutrients—Data for macronutrients in the fjord during the period of this study were kindly provided by Göteborgs och Bohus läns Vattenvårdsförbund. They have been sampling at Björkholmen on a monthly basis since 1990 as part of a large-scale monitoring program (Water Quality Association of the Bohus Coast 1997).

Cu speciation by CLE-CSV—CLE-CSV measurements were made with a Ecochemie μAutolab connected to a Metrohm VA 663 voltammeter used in the static mercury drop electrode mode. Instrument settings and protocols with salicylaldoxime (SA) were identical to those described by Campos and van den Berg (1994). Similarly, for benzoylacetone (bzac), experimental conditions were identical to that employed in Croot et al. (2000). Cu titrations were performed as follows. Each sample filtrate was divided into 20-ml aliquots in 125-ml Teflon bottles. These were spiked with different concentrations of Cu (0–80 nmol L^{-1}) and either SA (5–10 $\times$ 10^{-6} mol L^{-1}) or bzac (1–5 $\times$ 10^{-4} mol L^{-1}). The solutions were generally allowed to equilibrate for 3–6 h before analysis, by which time steady-state values were obtained. For analysis, 20 ml of solution was transferred to a Teflon sample cup and installed on the electrode, which was set to hanging drop mode. Anodic stripping voltammetry titrations were also made on several samples using the procedures outlined in Croot et al. (1999).

The total dissolved Cu concentration (Cu_T) in each sample was determined after ultraviolet (UV) oxidation of a 100-ml aliquot of seawater acidified to pH 2 with Ultrex HCl. A UV system, using a 1,200-W UV lamp, made by ACE Glass was employed. Samples were irradiated for 3 h in acid-cleaned quartz tubes. The sample pH was adjusted to 7.7 with isothermally distilled ammonia and *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer and Cu analyzed by CSV with 5 $\times$ 10^{-6} M SA. Replicate analysis typically gave a precision of 0.1 nmol L^{-1}.

SA (Aldrich) and bzac (Aldrich) required purification before use. This was accomplished by recrystallization in aqueous edatic acid (EDTA) solution (10^{-3} mol L^{-1}) followed by double recrystallization in Milli-Q to remove the EDTA. A solution containing 1 $\times$ 10^{-2} mol L^{-1} SA, or 5 $\times$ 10^{-2} mol L^{-1} bzac in methanol was used as a stock solution.

To determine ligand concentration and conditional stability constant data from Cu titrations, the fraction of Cu present as the $Cu(SA)_2$ complex at each point on the titration curve must be known. Therefore, the system must be calibrated accurately so that $[Cu(SA)_2]$ can be calculated from the peak current signal generated by the cathodic scan.

The peak current i_p is related to the concentration of $Cu(SA)_2$ in solution by Eq. 1.

$$i_p = S[Cu(SA)_2] \quad (1)$$

S is the sensitivity and is readily determined in UV-oxidized samples by standard additions of Cu. However, in natural samples, *S* must be determined from the linear portion of the titration curve when all complexing ligands are saturated in order to distinguish the effects of ligand competition, which does not affect *S*, from surfactant interferences, which do (van den Berg 1984). A third kinetic approach was also used throughout this work, in which Cu was directly added to a natural sample and analyzed immediately. In this case, the high concentrations of SA and bzac rapidly complex the added Cu; thus, *S* can be determined directly from the slope of current plotted against the concentration of added Cu. In general, there was very good agreement between the three methods used for obtaining *S* in this work.

For the present work, I was interested in the detection of strong copper-binding ligands, which workers in the field normally denote as L1. To achieve this aim, a scheme was developed that utilized multiple detection windows: 5 μmol L^{-1} SA, 2 μmol L^{-1} SA, and 200 μmol L^{-1} bzac and ASV. The conditional stability constants and ligand concentrations of all ligands in the sample are related by the following relationship as shown in Eq. 2 for the case of SA.

$$\frac{[Cu(SA)_2]}{[Cu_T]} = \frac{\alpha_{SA}}{1 + \Sigma K_i L_i + \alpha_{SA}} \quad (2)$$

K_i and L_i are the conditional stability constant and concentration of the *i*th natural ligand, respectively. The term $\Sigma K_i L_i$ represents the side-reaction coefficient for the naturally occurring ligands, where α_{SA} is the side-reaction coefficient for the $Cu(SA)_2$ complex ($\alpha_{SA} = \beta_2[SA]^2$), which was determined against model ligands (EDTA, diethylenetriaminepenta-acetic acid [DTPA]). The side-reaction coefficient (α) for all naturally occurring ligands (including inorganic ligands) is related to free cupric ion concentration by the relationship in Eq. 3.

$$\alpha = \frac{\Sigma CuL_i}{[Cu^{2+}]_f} = \frac{[Cu]_T - [Cu(SA)_2]}{[Cu^{2+}]_f} = 1 + \Sigma K_i L_i \quad (3)$$

Data in this study were analyzed with a ligand model that was a nonlinear fit to a Langmuir adsorption isotherm. This model has been described previously by Gerringa et al. (1995). Those workers made a convincing case from a statistical perspective for selecting a nonlinear fit over linearization plots, such as van den Berg/Ruzic or Scatchard plots. The single ligand model is derived from

$$K = \frac{[CuL]}{[Cu^{2+}]_f[L]_f} \quad (4)$$

where

$$[L] = [L]_f + [CuL] \quad (5)$$

Rearranging Eqs. 4 and 5 yields a reciprocal Langmuir isotherm.

$$\frac{[CuL]}{[Cu_f^{2+}]} = \frac{K[L]}{1 + K[Cu_f^{2+}]} \quad (6)$$

I used the program Origin 4.1 (Microcal software) to solve Eq. 6 for K and [L] by nonlinear regression analysis (Levenberg–Marquardt algorithm) with $[Cu^{2+}]_f$ as the independent variable and, $\Sigma K_i L_i/[Cu^{2+}]_f$ as the dependent variable(s). Estimates of the errors in K and [L] were obtained from this procedure. For simplicity, the weaker inorganic ligands present in the media (such as carbonate and chloride), are combined to form an inorganic side-reaction coefficient, α_I. For simplicity, a value of 26 was used for α_I throughout this study (Byrne et al. 1988). In the case when more than one distinct ligand class is present, the more correct form of the Eq. 6. is thus

$$\frac{[CuL_i]}{[Cu_f^{2+}]} = \Sigma K_i L_{i(i>1)} + \frac{[L_1]K_1}{1 + K_1[Cu_f^{2+}]} \quad (7)$$

where $\Sigma K_i L_{i(i>1)}$ is the side-reaction coefficient for the weaker ligands and K_1 and L_1 represent K and L in Eq. 6. Equation 7 was also solved using nonlinear regression in the cases where more than one ligand class was clearly present.

Values of α for the CSV ligands were calibrated against EDTA in UV-irradiated seawater over a range of salinities. In water of salinity 35, for 2 μmol L^{-1} SA, a value of log $\alpha_{SA} = 3.75 \pm 0.22$ was determined; this compares well to that found in an early study (log $\alpha_{SA} = 3.85 \pm 0.20$; Campos and van den Berg 1994). Side-reaction coefficients were corrected for the salinity. For SA, this was accomplished by using the relationship found by Campos and van den Berg (1994), whereas for bzac, corrections were applied by using thermodynamic data for the closely related compound acetylacetone (Martell and Smith 1982). At the lowest salinities found in this study (20–24), this correction amounted to roughly a 10% increase in α_{SA} and a 25% increase in α_{bzac}. All experiments were run at room temperature 22–23°C; no correction was made to side-reaction coefficients for in situ temperatures.

Calibration of the detection system was performed by recovery experiments in KMF seawater. Total Cu was verified by making standard additions of nanomolar amounts of Cu to natural KMF seawater. Measured recoveries during the period of this study were 97 ± 5% ($n = 12$). Validation of the detection system was performed using a dual-model ligand addition. Model ligands similar to class 1 (DTPA, log $K' = 13.73$, 25 nmol L^{-1}) and class 2 (EDTA, log $K' = 10.96$, 50 nmol L^{-1}) were added to UV-irradiated KMF seawater (salinity 26). Application of the same procedure as used for the determination of natural ligands gave the following results in good agreement with the theoretical values: log $K_1 = 13.6 \pm 0.4$, $L_1 = 24 \pm 3$ nmol L^{-1}, log $K_2 = 10.8 \pm 0.3$, $L_2 = 48 \pm 6$ nmol L^{-1}. The detection limit for L_1 and L_2 in this study is estimated as 1.2 and 3 nmol L^{-1}, respectively.

Error analysis for Cu speciation—The main focus of this study was to determine any changes in Cu speciation over a seasonal cycle. This required a rigorous error analysis of the Cu speciation data so as to reliably determine whether any changes were significant. Previous studies, particularly those using only the van den Berg/Ruzic linearization, have used a simplified error analysis and have not taken into account the errors involved in the linearization of the data, nor included the errors arising from measurement, leading to an underestimate of the true error. In the present study, the use of multiple detection windows precludes the use of such linearization schemes. For this study, error analysis was derived using procedures developed in Miller and Miller (1993). The results from the nonlinear regression analysis provide standard deviations for $K_i(\sigma_{K_i})$ and $L_i(\sigma_{L_i})$. The standard deviation for any log K, is then Eq. 8.

$$\sigma_{\log K} = \left|\frac{\sigma_K}{K}\right| \quad (8)$$

For the present study, the error in α_{CuL_i} is derived from the elements of Eq. 6, and when $[Cu]_f \ll [CuL_i]$ can be simplified to Eq. 9.

$$\frac{\sigma_{CuL_i}}{\alpha_{CuL_i}} = \sqrt{\left(\frac{\sigma_{K_i}}{K_i}\right)^2 + \left(\frac{\sigma_{L_i}}{[L_i]}\right)^2} \quad (9)$$

The standard deviations of each σ_{CuL_i} are combined to give σ_α, the standard deviation of the overall side-reaction coefficient α.

$$\sigma_\alpha = \sqrt{(\alpha_{CuL_1}\sigma_{CuL_1})^2 + (\alpha_{CuL_2}\sigma_{CuL_2})^2} \quad (10)$$

Using Eq. 3, this then allows an error estimate for pCu (pCu $= -\log[Cu_f]$).

$$\sigma_{pCu} = \sqrt{\left(\frac{\sigma_\alpha}{\alpha}\right)^2 + \left(\frac{\sigma_{Cu_T}}{[Cu]_T}\right)^2} \quad (11)$$

Microplankton identification—Subsamples for microplankton identification were obtained directly from unfiltered water collected from the GoFlo bottle. The cells were observed as soon as possible after collection, typically within 1–12 h afterwards. Microplankton samples were not preconcentrated, to avoid rupturing any delicate species. Sedimentation chambers (10 ml Utermohl) were used for the phytoplankton cell counts. The sedimentation chambers were left to settle for a day, and then the cells were counted on a Leica DM IL inverted microscope using a ×20/0.40 objective. The counting included an initial general assessment of the chamber (at ×20 magnification) in order to identify most of the species contained within the funnel. A subsequent linear transect was performed to count the number of cells that fell within the field of view. A Graticules LTD measuring slide was used to measure the field of view.

Picoplankton abundance/identification—Water samples for picoplankton abundance were obtained after prefiltration through a 2-μm (Nuclepore) filter and fixed with cold glu-

taraldehyde to a final concentration of 1% and stored at 4°C. Upon return to the laboratory, the sample was filtered through a black stained polycarbonate filter (Nuclepore) with a pore size of 0.2 μm using a vacuum of <100 mm Hg. Filters were mounted in fluorescence-free immersion oil and were counted immediately. Organisms were observed at ×1,000 magnification using a Leitz Dialux epifluorescence microscope equipped with a 50-W mercury lamp and filter sets for UV-blue and -green excitation. Eukaryotic picoplankton were counted using blue excitation light, and *Synechococcus* were counted in green light (Kuylenstierna and Karlson 1994).

Results and discussion

Seasonal forcings—There was a strong seasonal gradient in sea surface temperature during this study, with surface temperatures ranging from −1 to 22°C (Fig. 2). Some surface ice was encountered close to KMF during January 1997 and appeared to be related to freshwater freezing over the calm seawater. The deep waters of the fjord (<50 m) remained at 6°C throughout this period. Water and air temperatures during summer 1997 were the highest on record since 1860 (Water Quality Association of the Bohus Coast 1997), with the air temperatures in August 4.5°C higher than normal. This led to higher than normal seawater temperatures during August and September 1997 when compared to data from the previous 10 yr (Lindahl et al. 1998).

There was little horizontal variation in depth profiles along the fjord, with the exception that surface salinities were typically lower at Björkholmen, close to the main freshwater inlet to the fjord, and increased seaward. There was negligible tidal variation in the surface salinity. Mixed layer thickness was always <15 m because of the strong pycnocline induced by low salinity (<28) Baltic water at the surface. Under conditions of high runoff into the fjord, mixed layers could be as shallow as 2–5 m. Below the mixed layers in the water of Skagerrak origin, salinity was more uniform (33.5–34). During winter 1996/1997, no deep-water exchange took place, resulting in severe oxygen deficiency below 80 m in the fjord (Liljebladh and Thomasson 2001).

Mixed-layer chlorophyll *a* (Chl *a*) concentrations over the survey period ranged from 1.5 to 8 mg m^{-3}, with highest concentrations in February 1997 (Fig. 2). The bloom in February was somewhat exceptional because it began in the Kattegat between 19 January and 25 January, reaching 6–13 mg m^{-3} Chl *a* (Water Quality Association of the Bohus Coast 1997). The weather at this time was very calm and sunny, leading to the spring bloom starting 2 months earlier than usual. In most years, there are low levels of chlorophyll during the summer months in the fjord (Lindahl and Hernroth 1983). The small bloom we encountered in May 1997 was restricted to a shallow surface layer of freshwater runoff mixing with Baltic water. During this survey, we did not encounter the exceptionally high chlorophyll concentrations (>10 mg m^{-3}) that have been observed in early studies (Lindahl and Hernroth 1983; Water Quality Association of the Bohus Coast 1997); this appears to be due to the unusual climactic conditions.

Macronutrients—Macronutrient data (Water Quality Association of the Bohus Coast 1997) for the period of this study is shown in Fig. 3. A strong seasonal pattern can be seen for phosphate in the surface waters, with significant surface concentrations only during the winter. Phosphate has a clear maximum in the winter of 1996/1997 and is depleted through the spring and summer to a minimum at the beginning of autumn. The early spring bloom of February 1997 is responsible for much of the drawdown, leading to lower than normal chlorophyll levels during March and April.

Nitrate and silicate (Fig 3) show a similar trend to phosphate, except during periods of strong runoff when surface concentrations were significantly enhanced. Mesocosm studies in Gullmar Fjord have shown that a low N : Si (1 : 1) favors diatom growth, whereas a high N : Si (4 : 1) favors small flagellates (Schöllhorn and Graneli 1996). These authors hypothesized that eutrophication and increased nitrogen discharges to the fjord were important factors behind the increase of flagellate/dinoflagellate blooms observed in the fjord during the last 20 yr. During the present study, the NO_3 : Si in the water column ranged from 0.01–1.7 (mean 0.55 ± 0.35), lower than has been reported for Gullmar Fjord in early studies (Schöllhorn and Graneli 1996), perhaps indicating a reduction in the nitrogen load from runoff during 1996–1997.

The seasonal progression in macronutrient concentrations is also clearly seen in depth-integrated (0–40 m) values for nitrate and chlorophyll (Fig. 4, upper panel). The nitrate inventory in the upper water column increases to a maximum in winter and then is depleted during the spring and summer. The early spring bloom could have altered the usual ecosystem structure by the reduction of nutrient stocks earlier than usual. The depth-integrated chlorophyll levels during this study are similar to those found in earlier work in the fjord (Lindahl and Hernroth 1983). There was also a strong seasonal signal seen in the depth-integrated (0–40 m) inventories of NO_3 : Si and NO_3 : PO_4 (Fig. 4, lower panel), with maximum ratios in March/April 1997. NO_3 : PO_4 was less than the typical Redfield ratio (16 : 1) (Redfield et al. 1963) for most of the year, perhaps indicating an overall nitrate-limited system, as has been suggested by other workers (Lindahl and Hernroth 1983).

Algal community structure during the survey—At the beginning of this survey in autumn 1996, the phytoplankton community was dominated by dinoflagellates, with *Ceratium* species very abundant, as has been seen in other years (Lindahl and Hernroth 1983). The spring bloom in January/February 1997 was dominated by diatoms (*Chaetoceros* sp., *Skeletonema costatum,* and *Thalassiosira nordenskioldi*). In March, diatoms were still dominant, but some *Phaeocystis* was also present. During May and June, the phytoplankton community was more diverse, with dinoflagellates (*Ceratium longipes, Prorocentrum* sp.) and diatoms present (*Leptocylindrus danicus, Chaetoceros* sp). By July, prymnesiophytes were also significant (*Emiliani huxleyi* and *Chrysochromulina* sp). In autumn 1997, dinoflagellates (*Prorocentrum micans,* many *Ceratium* sp., *Gymnodinium* sp., and some *Dinophysis* sp.) had again become dominant as in 1996, but diatoms (*L. danicus, Chaetoceros* sp., *Proboscia alata*) were

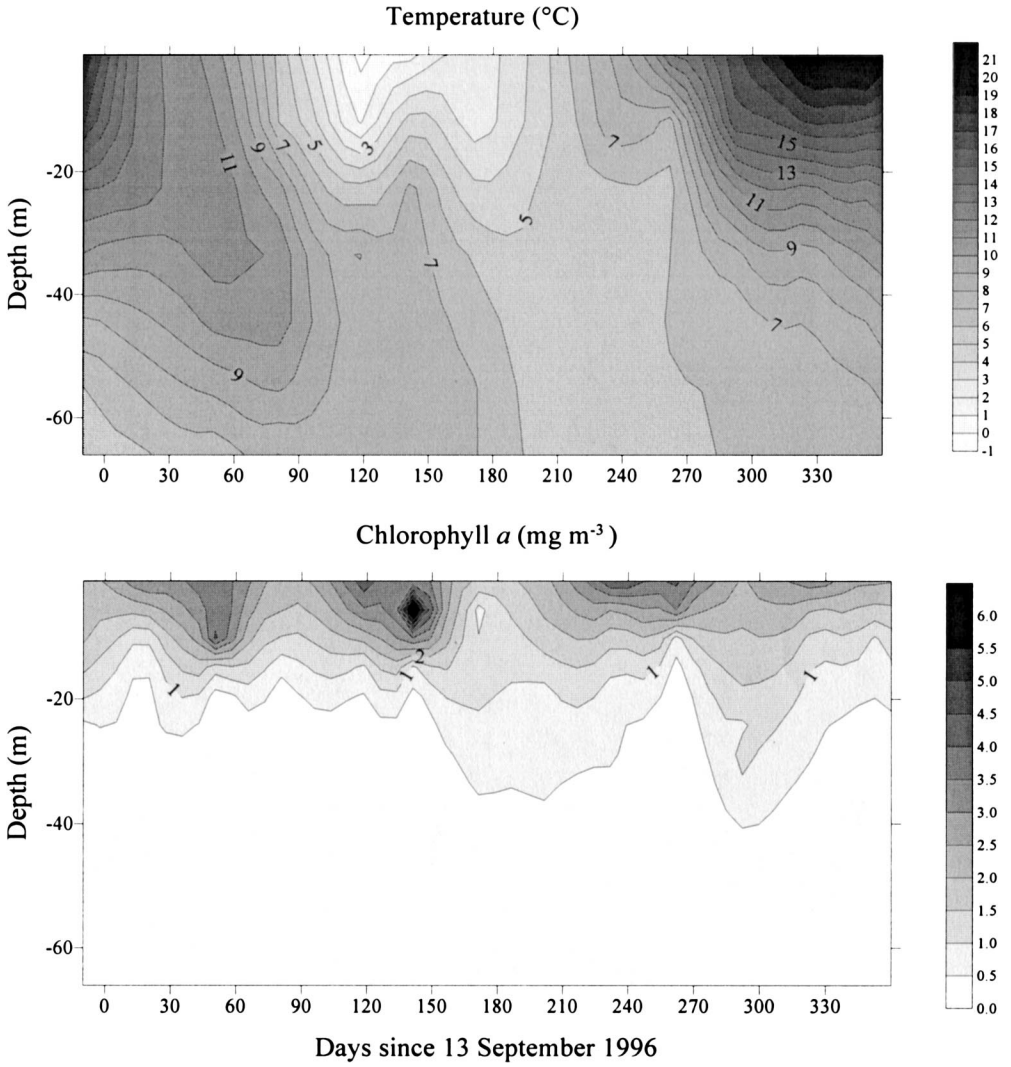

Fig. 2. Temperature and Chl *a* at Björkholmen in Gullmar Fjord (data from the Göteborgs och Bohus läns Vattenvårdsförbund) from September 1996 until September 1997.

still significant. Despite the low silicate concentrations in the upper 20 m (Fig 3), diatoms were apparently utilizing episodic pulses of silicate that were associated with runoff from the surrounding countryside.

Phycoerythrin-rich cyanobacteria of the type *Synechococcus* (Table 2) were found at all times with low numbers in the winter (4×10^5 cells L^{-1}) and maximum values ($\sim 1 \times 10^8$ cells L^{-1}) in summer, consistent with early studies in Gullmar Fjord (Kuylenstierna and Karlson 1994; Tiselius and Kuylenstierna 1996) and the Swedish west coast (Karlson and Nilsson 1991; Karlson 1995). *Synechococcus* numbers increased dramatically in surface waters during the surveys of May 1997 (range 4.1–31 $\times$ 10^6 cells L^{-1}). The timing of this increase in *Synechococcus* numbers was almost a month earlier than that reported by (Kuylenstierna and Karlson 1994) and is probably related to the early warming of the surface waters during 1997. The global distribution and appearance of *Synechococcus* has been shown to be strongly correlated to seawater temperature (Li 1998), and for the Skagerrak region, the 10°C isotherm is a useful guide for significant numbers of *Synechococcus* in the water column (Kuylenstierna and Karlson 1994). *Synechococcus*-type cells observed during this study from January and March 1997, when temperatures were <7°C, were few in number and weakly fluorescent, which could indicate a state of dormancy or inactivity.

Copper concentrations in Gullmar Fjord—Dissolved Cu (*D*Cu) in the fjord ranged from 2.4 to 18.5 nmol L^{-1} (Table 2). Highest concentrations were found in surface water with salinities lower than that typical of Baltic water, consistent with local runoff into the fjord. In this study, seawater of Skagerrak origin contained 2–4 nmol L^{-1} dissolved Cu, whereas water of Baltic origin was higher at 4–12 nmol L^{-1}, consistent with early work in the Kattegat and Skagerrak (Magnusson and Westerlund 1983). There have been few studies of Cu in Gullmar Fjord. Westerlund et al. (1986) reported bottom water Cu concentrations of 5.5–7.9 nmol L^{-1}. They found a significant benthic flux to bottom waters

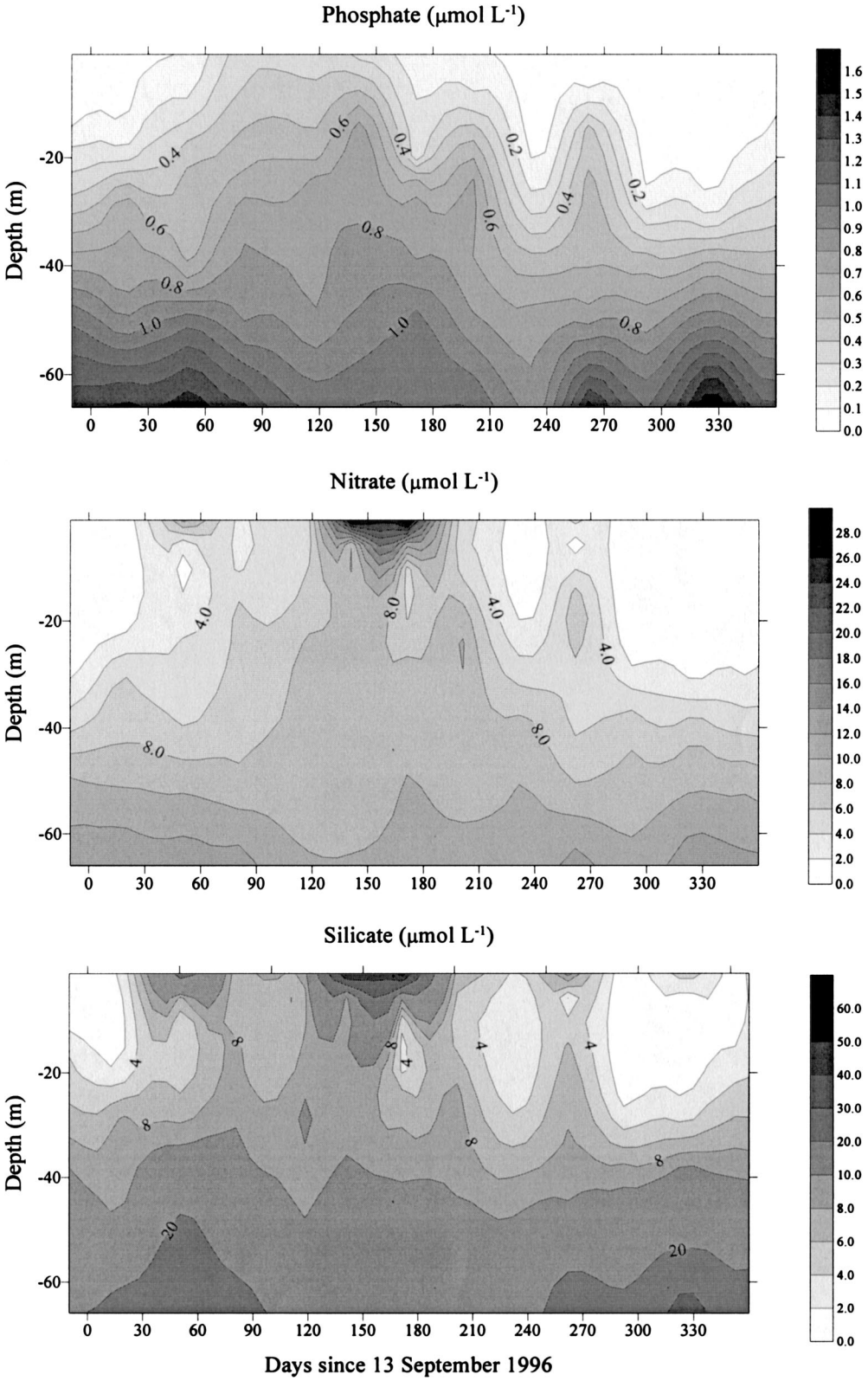

Fig. 3. Phosphate, nitrate, and silicate at Björkholmen in Gullmar Fjord (data from the Göteborgs och Bohus läns Vattenvårdsförbund) from September 1996 until September 1997.

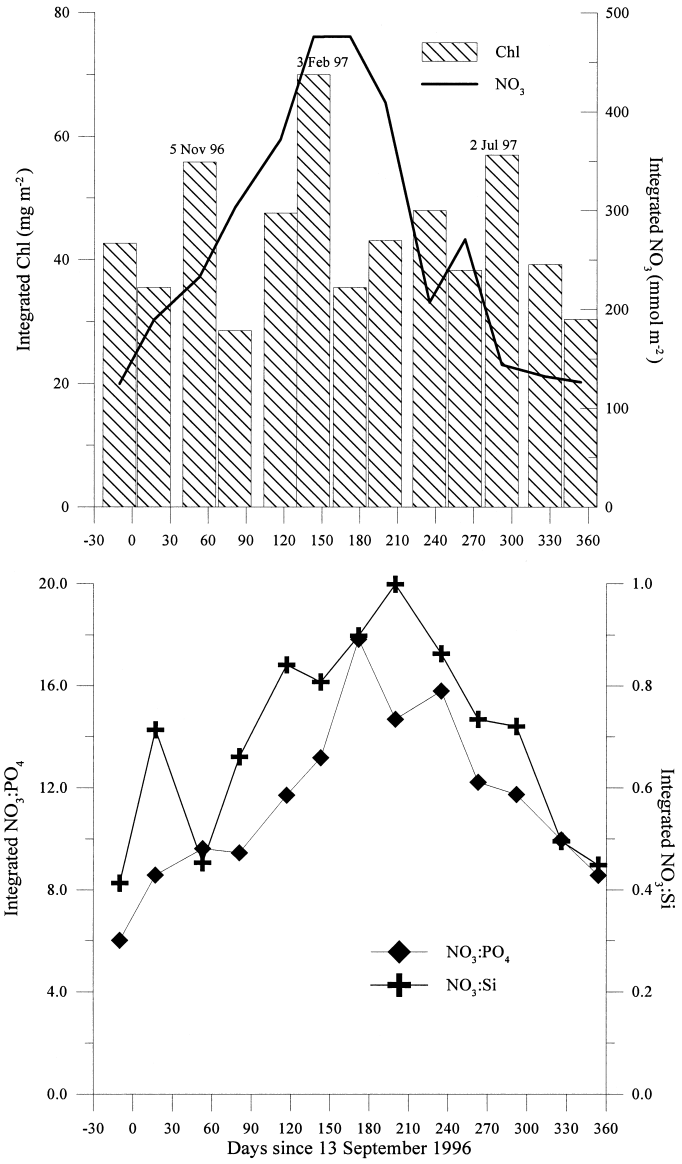

Fig. 4. (Upper panel) Depth-integrated (0–40 m) Chl *a* and nitrate at Björkholmen in Gullmar Fjord (data from the Göteborgs och Bohus läns Vattenvårdsförbund) from September 1996 until September 1997. (Lower panel) Nutrient ratios ($NO_3:PO_4$, $NO_3:Si$) from depth-integrated inventories (0–40 m) at Björkholmen.

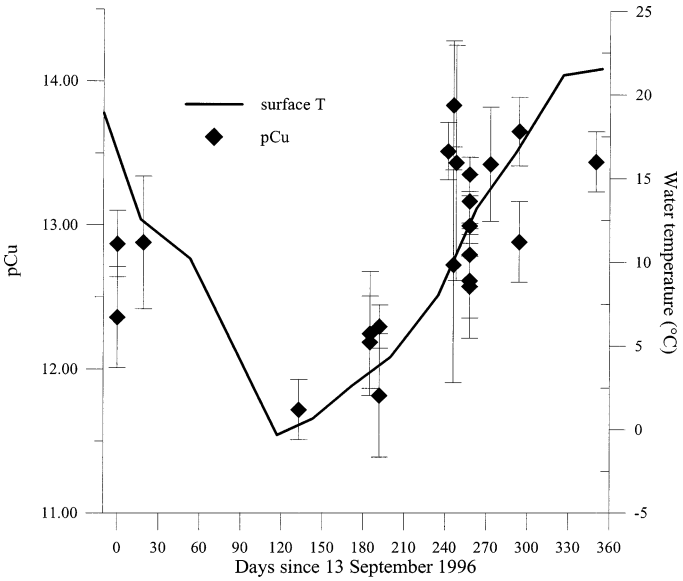

Fig. 5. Values of pCu (all data) and surface water temperature in Gullmar Fjord during the period September 1996 to September 1997. The errors for pCu are at the 95% confidence level.

of Cu (118 nmol m^{-2} d^{-1}) during the autumn, with lower rates in the winter.

Seasonal changes in Cu speciation—Copper speciation in Gullmar Fjord was dominated by organic complexation (over 99.8%) throughout this study (Table 2). Strong class 1 Cu binding ligands were detected in autumn 1996, both in Gullmar Fjord and in the Skagerrak. No strong ligand was detected during the winter or early spring. High concentrations of L1 were found in surface waters from late spring (May) until autumn (August) 1997, with the exception of the survey on 29 May. At that time, surface water salinities were lower (~21) than normal because of a strong riverine outflow, as evidenced by a muddy brown appearance. *Synechococcus* cell numbers in the near-surface waters were also an order of magnitude lower at this time than 10 d early.

The calculated free copper concentration ($pCu = -\log[Cu]_f$) over the course of this study is shown in Fig. 5. The values found in this study are consistent with other coastal studies (Moffett et al. 1997; Tang et al. 2001). There is an apparent seasonal signal in pCu, with low values (high $[Cu]_f$) in the winter and high values (low $[Cu]_f$) in summer. Comparison between surface-water temperature and pCu during this work highlights this seasonal periodicity (Fig 5). There is insufficient data however to determine with any statistical significance the lead/lag time between in situ temperature and pCu or $[Cu]_f$. Earlier time series studies by Coale and Bruland (1990) and Moffett (1995) at oligotrophic open-ocean stations saw a constant presence of L_1 and only small fluctuations in pCu from season to season. This study is the first to look at copper speciation over the course of an annual cycle in a coastal environment in detail and confirms the suggestion by Brand et al. (1986) that seasonal cycles should exist for copper speciation.

Earlier studies on Cu speciation in open ocean waters have found low concentrations of L_1 ligands, in an almost 1:1 relationship with ambient dissolved Cu (Coale and Bruland 1988, 1990; Moffett et al. 1990; Moffett 1995). In the present study, this 1:1 relationship is also seen, with the exception of data from 13–19 May, where an excess of L_1 is found (Fig. 6). An excess of L_1 was previously reported in July 1997 in the central Skagerrak (Croot et al. 2002) and has been seen in Vineyard Sound (Moffett et al. 1997), where it was suggested it might be derived from a terrestrial source. During 13–19 May, a strong outflow of terrestrial material was observed in the upper 1–2 m of the water column, but this was in a different location from the site we obtained our samples. On this date, however, attempts to determine Cu speciation in both the upper 1 m of the fjord

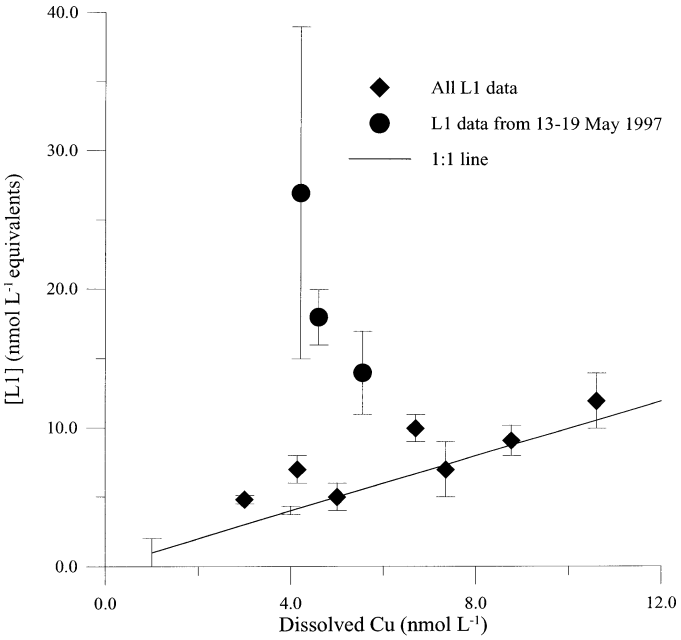

Fig. 6. Relationship between $[L_1]$ (defined where $\log K_1 > 12.5$) and dissolved Cu concentrations during this study.

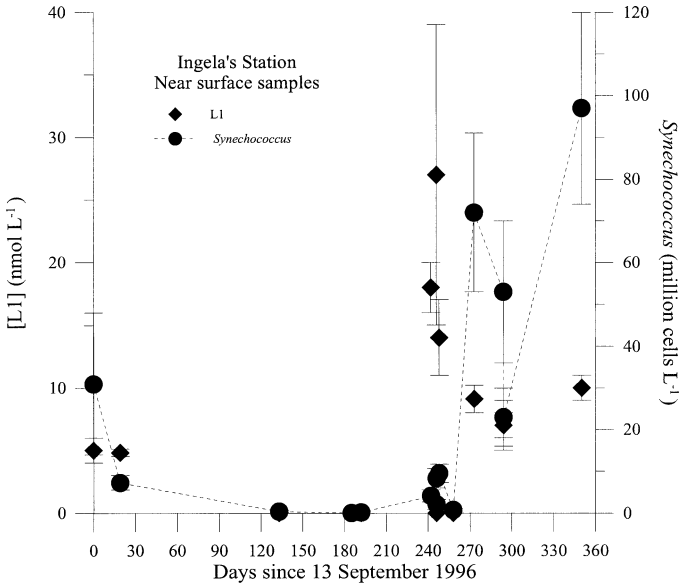

Fig. 7. Relationship between $[L_1]$ and *Synechococcus* abundance in near-surface samples at Ingela's station.

and in the river water itself were unsuccessful because of overwhelming interference from surfactants. An alternative explanation/source could be that the excess of L_1 reflects an overstimulated response by the biota to Cu stress. *Synechococcus* numbers were beginning to increase as the water warmed and $[Cu]_f$ might have been high enough to affect the growth rate. This high $[Cu]_f$ could be caused by weakly bound Cu in freshwater or in the Baltic current mixing with the Skagerrak water where the *Synechococcus* were. During the time it takes for the system to come to thermodynamic equilibrium, the *Synechococcus* would be exposed to an elevated $[Cu]_f$. The Gullmar Fjord, and coastal areas in general, can be a dynamic environment for $[Cu]_T$, with obvious implications for $[Cu]_f$. For the open ocean, where $[Cu]_T$, and perhaps $[Cu]_f$, are more stable, a 1 : 1 relationship between L_1 and $[Cu]_T$ could be maintained more easily.

A further station was occupied at Alsbäck/Djuphålan in late summer 1998 (Table 2) in an effort to examine Cu speciation in the deep waters of the fjord. No L_1 ligands were detected at 70 m, consistent with the lack of significant *Synechococcus* abundance because of the absence of light and consistent 6–7°C. There was a slight vertical trend in pCu, which was also seen in pFe, suggesting a benthic source for iron-binding ligands (Croot and Johansson 2000). Skrabal et al. (1997) have shown that sediments can be a significant source of Cu complexing ligands, with ligand fluxes 6–40 times in excess of the dissolved Cu flux. This flux of ligands from the deep water of the fjord might control Cu speciation in the deep water. The Cu flux from the bottom sediments in Gullmar Fjord during 1997 might have been enhanced by the low O_2 levels in the overlying bottom waters (Liljebladh and Thomasson 2001), suggesting that there could also exist a seasonal cycle in deep-water Cu speciation in the fjord (Westerlund et al. 1986).

Possible sources and sinks of Cu complexing ligands—In the present work, the synchronicity of the appearance of L_1 and *Synechococcus* point strongly to these cyanobacteria as an L_1 source (Fig. 6). Interestingly, there was no correlation between bulk chlorophyll and L_1 or pCu, indicating that only a small part of the phytoplankton community might be responsible for L_1 production. Our data are consistent with the hypothesis that *Synechococcus* is a strong candidate for L_1 production (Moffett et al. 1990; Moffett 1995; Moffett and Brand 1996). L_1 was only found in Gullmar Fjord in regions of, and during times of, significant ($>1 \times 10^7$ cells L^{-1}) *Synechococcus* abundance (Fig. 7). As yet, no eukaryotic phytoplankton species has been found to produce class 1 ligands when grown under elevated Cu concentrations (Croot et al. 2000). Currently, only *Synechococcus* has been found to produce L_1-type ligands, although the prochlorophyte *Prochlorococcus* might also be a source (Croot et al. 2000), but has yet to be found in the Skagerrak or Gullmar Fjord. In this study, we did not find a 1 : 1 correlation between L_1 concentration and *Synechococcus* abundance, in agreement with data from the adjacent Skagerrak in July 1997 (Croot et al. 2002). This could also imply that L_1 is present throughout the year but is only present at concentrations above the detection limit of the techniques used here during periods of high *Synechococcus* abundance. Equally, however, other organisms present at this time could be responsible—in particular, heterotrophic bacteria, which were not studied in this work. Presently, there is no evidence for production of L_1 by any species of heterotrophic bacteria, although it cannot be discounted. Of the eukaryotic species present during the period that L_1 was detectable, there is no strong candidate for L_1 production, as many of the algae present (*Chaetoceros* sp., *Ceratium* sp., and *Prorocentrum* sp.) are reasonably Cu tolerant and known to produce weaker L_2 ligands (*see below*). The only sink for L_1-type ligands

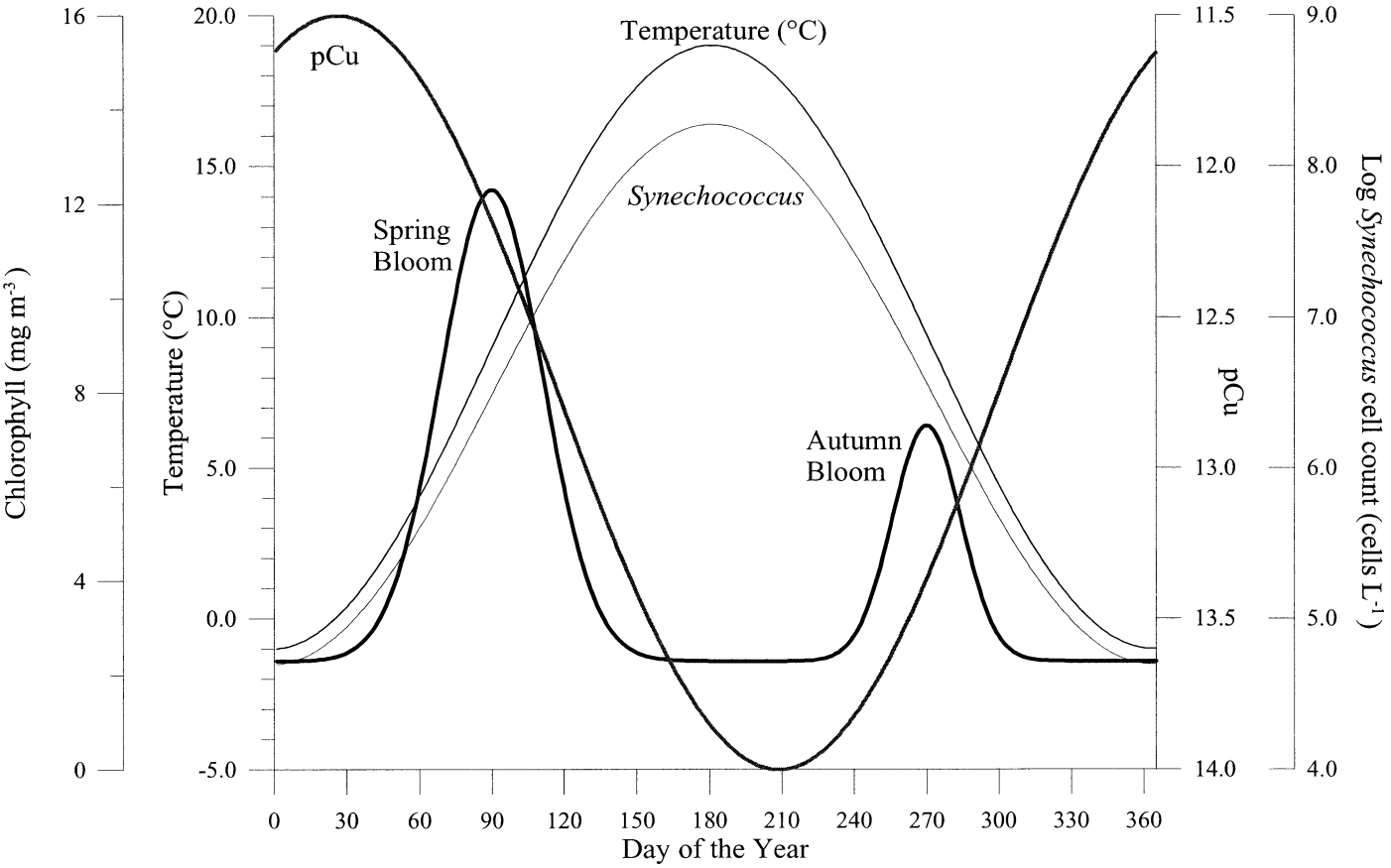

Fig. 8. Idealized representation of the seasonal cycles affecting Cu speciation in the Gullmar Fjord.

known presently is from destruction/alteration by sunlight (Moffett et al. 1990; Croot et al. 1999).

Eukaryotic phytoplankton (Zhou and Wangersky 1989; Lage et al. 1994; Leal et al. 1999; Croot et al. 2000) and macroalgae (Gledhill et al. 1999) have been found to produce extracellular Cu complexing ligands in response to Cu stress. In general, it appears that the organisms producing the strongest chelators are the most sensitive to Cu_f. The growth rate of several dinoflagellate species is reduced at a pCu $\approx$ 11 (Brand et al. 1986). This includes *Prorocentrum* sp. similar to those identified in Gullmar Fjord. Laboratory strains of *P. micans*, a species that was present in Gullmar Fjord in autumn 1997, have been found to produce a moderate Cu complexing ligand (Croot et al. 2000), but perhaps not as a Cu tolerance mechanism. Instead, it might be related to phosphate stress as seen in another *Prorocentrum* sp. (Dyhrman and Palenik 1999). Another dinoflagellate species, *Amphidinium carterae* produces slightly weaker Cu complexing ligands (log K = 12) when under Cu stress in a 1 : 1 relationship similar to *Synechococcus* (Croot et al. 2000). Diatoms produce Cu complexing ligands during different stages of growth (Zhou and Wangersky 1989; Croot et al. 2000), with one strain of the common coastal diatom *S. costatum* (also present in the fjord) found to produce Cu chelating ligands with log K $\approx$ 12 (Croot et al. 2000). It is clear then that in Gullmar Fjord, the phytoplankton community present throughout the year was a major source of Cu complexing ligands, produced either actively in response to Cu stress or passively as a response to nutrient stress or some other factor.

During May 1998, a large bloom of the raphidophyceae *Chatonella* sp. occurred in the Kattegat and along the Swedish west coast. From 14–18 May 1998, a station at Valö, in the Göteborg archipelago, was occupied. Observations showed an increase in thiol-like voltammetric peaks after the maximum extent of the bloom, consistent with release of glutathione and other thiol compounds from decaying cells (Leal et al. 1999). Surfactant compounds, as identified by AC voltammetry, increased during the bloom to a maximum just prior to the end of the bloom. No Cu complexing ligands of class 1 were detected at any time during the bloom. In the growth phase of the bloom, concentrations of L_2 slowly increased (log K = 12.2), and at the senescent stage, concentrations of L_2 doubled, mostly from a contribution from a weaker component (log K = 10.6). The trends for Cu ligands were also seen for Fe binding ligands, which increased at the end of the bloom (Croot and Johansson 2000).

Throughout the year, there was a high concentration (14–122 nmol L^{-1}) of L_2 ligands present that maintained pCu around 12, if no L_1 was present. There was some seasonality in the concentration of L_2 ligands, with the highest concentrations at the end of the spring bloom in March 1997 and the lowest concentration in July 1997 when nutrients were depleted. Currently, we have little or no information on the

chemical characteristics and functionalities present in L_2-type ligands. In recent years, a few candidate molecules have been proposed. Several workers have suggested that a possible source of L_2-type ligands are thiols from phytoplankton (Leal et al. 1999; Tang et al. 2001). Throughout this study, there were interferences with the CSV analysis from ''thiol-like'' compounds. In some cases, the initial peak for the thiol split on addition of Cu, indicating the initial peak was from the reduction of the Hg–thiol complex (the free thiol) and was thus most likely not complexed by Cu in the seawater. In the present work, the estimated thiol-like concentration, from comparison with glutathione, was always less than the measured L_2. Overall however, thiols could be a substantial part of the class 2 ligands present in seawater, but it remains an analytical challenge to determine this by nonpolarographic techniques because the strong reaction between the thiol S and the Hg electrode might bias these results. Another possible candidate for L_2 is domoic acid, which can reach up to 100 nmol L^{-1} (Rue and Bruland 2001) and is produced by toxigenic species of the pennate diatom *Pseudo-nitzschia*. During the present study, I observed a few *Pseudo-nitzschia* sp., especially in autumn 1997, so this source is possible, although the measured log K (10.3) for domoic acid (at salinity 35) is low. Bacterial uptake is the most likely sink for both thiol compounds and domoic acid.

The results gained from the present study point to a seasonal cycle in Cu speciation in Gullmar Fjord, which might be driven by L_1 production by *Synechococcus*. Figure 8 illustrates an idealized picture of these processes in Gullmar Fjord, by which temperature controls *Synechococcus* abundance, which in turn is related to L_1 production and the lowering of Cu_f. The real picture is more complicated than this, and it is clear that further study is needed on the chemical composition of L_1 and L_2 ligands, and more robust analytical techniques are required to determine the specific ligands that make up the various classes. The overall aim is to identify both the molecule responsible and the phytoplankton source. This might be accomplished by a combination of genomics, proteomics, analytical chemistry, and microscopy.

This study has demonstrated that a seasonal cycle exists in Gullmar Fjord for Cu speciation and that it appears to be driven by the seasonal abundance of *Synechococcus* in the fjord. Cu speciation in the surface waters of the fjord is dynamic on the same time scales of water mass movement, but a seasonal cycle is still clearly apparent. During winter in the fjord, Cu complexation was apparently controlled by ligands produced in the course of the life cycle of diatoms and dinoflagellates.

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